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Analysis of polyphenolic and protein content in craft and industrial beers

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When you make the finding yourself
even if you're the last person on
Earth to see the light – you'll never forget it.
Carl Sagan

First of all I want to thank my two tutors, Ph.D Concepción Lao Luque and Ph.D Francesc Xavier de las Heras Cisa, for all the time dedicated in the realization of this project and their advices.

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And finally, I want to thank my parents for being there, supporting and enduring these years.

Resum

La cervesa és una de les begudes més consumides al món. Tant la producció com el consum de cervesa artesanal mostren una tendència a l'alça, tractant de recuperar la tradició cervesera, mentre que la cervesa produïda industrialment és l'objectiu d'una major producció, garantint així la qualitat del producte final.

Aquesta és la raó per la quantitat de proteïnes i fenols totals es van calcular per a diferents tipus d'ambdues cerveses artesanals i industrials, per tal d'establir una comparació quantitativa i poder relacionar-los amb els ingredients que s'han utilitzat, el procés de producció i diferents variables que influeixen en el procés.

Una major quantitat de compostos bioactius s'ha obtingut de les mostres de cervesa artesanal que de les que es produeix industrialment. Les diferències s'atribueixen a les matèries primeres, tipus de processos i tipus de fermentacions. Els resultats es van comparar amb els resultats anteriors pels científics especialitzats en l’elaboració i l'anàlisi de la cervesa.

Summary

Beer is one of the most consumed drinks in the world. Both production and consumption of crafted beer show an upward trend, trying to recover the brewing tradition, whilst the industrially produced beer is aiming at a bigger production, thus ensuring the quality of the final product.

This is why the quantity of protein and total phenols were calculated for different types of both craft and industrial beers, in order to establish a quantitative comparison and to be able to relate them to the raw ingredients which have been used, the production process and other different variables which influence during the process.

A larger quantity of bioactive compounds has been obtained from the craft beer samples than from the industrially produces ones. The differences are attributed to raw materials, types of processes and types of fermentations. The results are compared with results made previously by specialized scientists

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Justification and objectives

1.1. Justification

Beer is one of the most consumed and socially accepted drinks in the world. Also, **brewing** is one of the most ancient processes in history, and every country has its own variations of the elaboration of the drink.

The production and consumption of **craft beer** has increased significantly in the last decade and is linked to the recovery of traditional processes of brewing and to the use of raw materials of better quality.

Industrial brewing is carried out on a large scale, seeking the highest production in the shortest possible time, using more raw materials trying to achieve an economic development. For this reason, it would be interesting to establish comparisons in the processing and in the raw materials that are used during the elaboration, to determine the differences that they can possess.

Numerous studies have shown the beneficial effects of moderate beer consumption, associated with its nutritional composition. Therefore, this bachelor's thesis analysis tries to determine the content of protein and total phenols in different samples of craft and industrial beers.

The objective is to determinate if the elaboration process influences the polyphenol and protein content and which among them gives beers with the highest levels of these compounds, by doing so, an increase in the interest of the consumers by their nutritional values.

1.2. Objectives

The main objective of this thesis is evaluating the effect of the type of elaboration process (craft or industrial) on the content of proteins and polyphenols in beers.

The intention is compare different elaboration processes (Lager, Dark Lager, Märzen and Pilsen) for both craft and industrial in order to establish which one gives beers the highest contents of proteins and polyphenols.

Also work with different methods of quantification in the laboratory, putting into practice the knowledge acquired during the degree, with the help of various articles and bibliographic references about beer and its characteristics.

2. Background

2.1. History

Beer was first produced by the **Sumerians** in southern Babylon at the end of 4.000 BC. Beer was one of the most important foods of the Babylonians' diet, being used as a currency in different transactions.

From Babylonians, **Egyptians** inherited the art of brewing (1), modifying the process, depending on the products they can afford. Babylonians used beer as an antiseptic substance to avoid plague contagions, and Egyptians improved the process techniques, obtaining a highest concentration of alcohol in the beverage, and selling them in their markets or as an offer to gods. (2)

With the arrival of the Christian religion, brewing was a process exclusive of the **monks** carried out in a lot of abbeys around Europe, most in **England, Belgium and Germany**. They had the information about antique processes of brewing and they had the knowledge of the main uses of aromatic plants.

At the XII century, beer was introduced into the upper classes of the European society of the time, where merchants and craftsmen appear and introduce a new ingredient, **hops**, taking a leading role in the process, giving body and flavor to beer.

With the new brewers, it had appeared the The ***Reinheitsgebot***, or also named as **The German Beer Purity Law of 1516**. It is the collective name for a series of regulations limiting the ingredients in beer in Germany and the states of the former Holy Roman Empire. (3)

Industrial revolution helped the brewing process increasing the efficiency and the market. **Louis Pasteur** in 1876 discovered **yeast,** and invented the **pasteurization process.** These discoveries made a huge decreasing of microbial infections in beer, avoiding losses in the process.

2.2. Definition

According to the **Official State Gazette** (4) (B.O.E according to its Spanish Acronym), **Industrial beer** is a drink produced by alcoholic fermentation of wort from barley malt (alone or mixed with other starch products) using water and selected yeasts. The fermentation process transforms sugars into alcohol by enzymatic digestion, and with the use of hops, derivatives and additives suffer different elaboration steps like filtration and pasteurization.

The Official State Gazette also refers to **Craft beer**, as an elaborated alcoholic beverage, in accordance with a determined standard of quality, a full developed process and under the direction a brewer master or artisan, combining experience and technologies, obtaining a final result acceptable to the legislation standards

In the following figure we can see the differences between the processes, industrial and craft, of brewing:

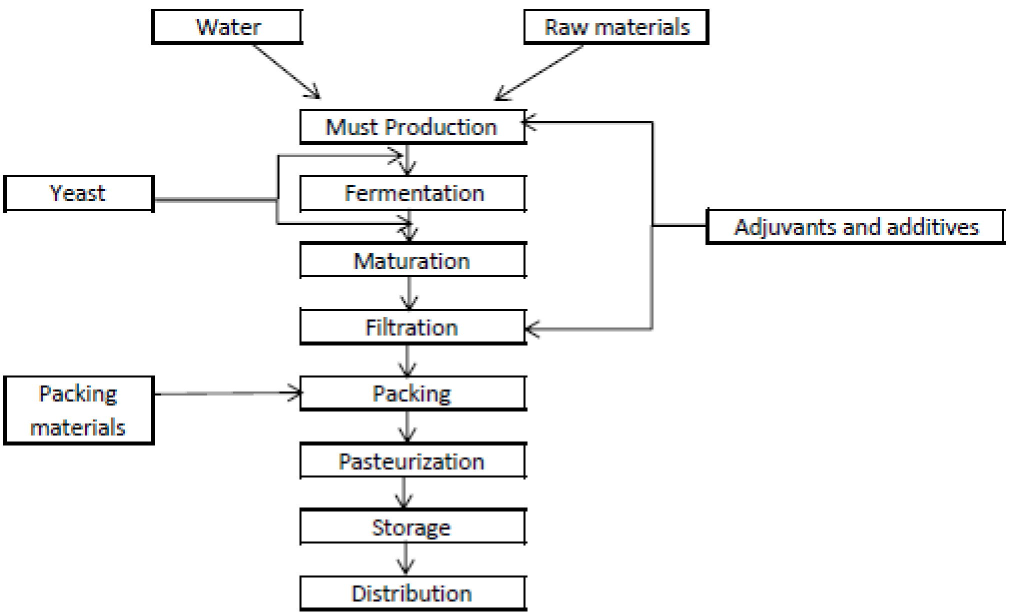


Figure 1: Flow diagram of industrial brewing

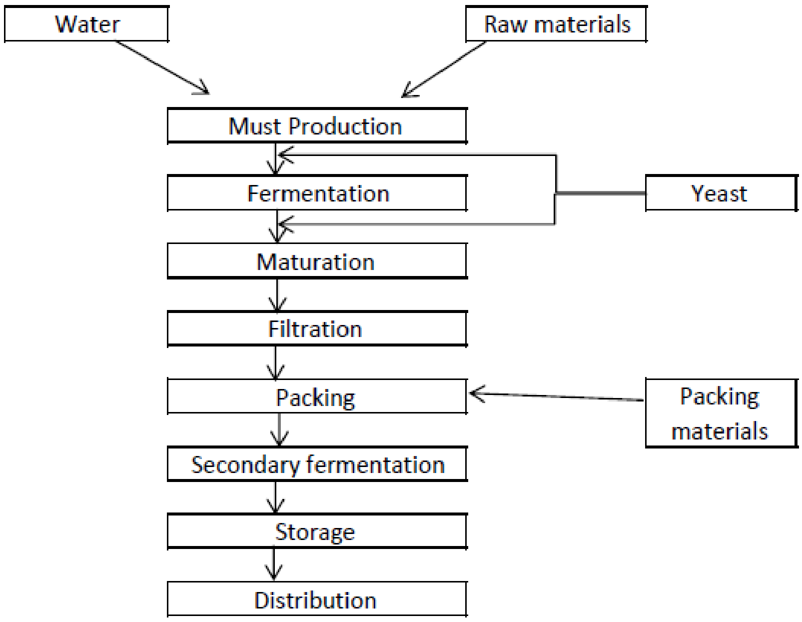


Figure 2: Flow diagram of craft brewing

The flow diagrams above summarize the process of brewing both craft and industrial beer, showing the main stages of the process. The diagrams show the complexity of the processes and their key stages to obtain a good product.

In the section: **Differences between industrial beers and craft beers**, we will explain in detail each of the stages of the process and in which they are characterized

2.3. Raw materials

Beer is made from water, barley malt, hops and yeast, and other type of additives.

The 95% of the beer, in terms of weight, is **water**. In water we can found a great diversity of elements diluted, like calcium, magnesium and bicarbonate.

Bicarbonate is a very important factor in the brewing process, because it can affect the **pH** of the beer, increasing it, giving as a result more **astringent** beers with a cleared color (blonde beers). Having problems with the pH can decrease the efficiency of the process, resulting a **bitter flavor** and highest sensibility to the development of lactic fermentations. When water has an adequate concentration of calcium ions, yeasts will flocculate better, helping the clarification of the wort and the beer. They also eliminate the excess of haze originated during the storage (1, 8).

Barley malt is the most used cereal in brewing because is the one who produces fewer complications during the process. Malt contains a lot of proteins, phenolic compounds, phosphates, vitamins and starch. From **starch** we can obtain the sugar transformed during the brewing process, by the catalysis produced by this group of enzymes: Phosphorylase, α-glucosidase and α and β-amilase. For brewing, we need the barley malt grains in good conditions, non-germinated and without parasites or infections. (1, 8)

Other cereals used in brewing are **corn, wheat and rice**. They are used mostly in the industrial processes because their prices are lower than malt.

Hop or *Humulus lupulus*, has the majority of the essential oils and resins who give beer a characteristic bitterness and aroma. Brewers use fecundated hops, because they contain more resins than virgin hops, intensifying the taste of the beer. (8, 12)

Yeast is a unicellular organism and it reproduce by budding. In brewing, they use the yeast who belong to the *Saccharomyces,* mostly by it capacity of fermentation in anaerobic conditions, producing ethanol and carbon dioxide in less proportion (8, 12). There are three types of yeasts, depending of their fermentation:

- **Top fermentation yeasts or Ale yeasts**: yeasts without flocculation capacity, with a hydrophobic behavior. Characteristic of Ale beers (9). The fermentation action takes place at the top of the fermenter. Ale yeasts like warmer temperatures below about 12ºC.(10)
- **Bottom fermentation yeasts or lager yeasts**: characteristic of lager beers (9). The fermentation action takes place at the bottom of the fermenter, at low temperatures (less than 10 ºC). They can be:
    - **Non flocculating yeasts**: they maintain their activity distributed in the fermenter and descend slowly at the end of the fermentation
    - **Flocculating yeasts**: they clump and descend to the bottom of the fermenter.
- **Spontaneous fermentation yeasts:** the microorganisms enter in a direct contact with the wort. It is produced in open air processes, giving beer a sour flavor and a higher graduation. (11)

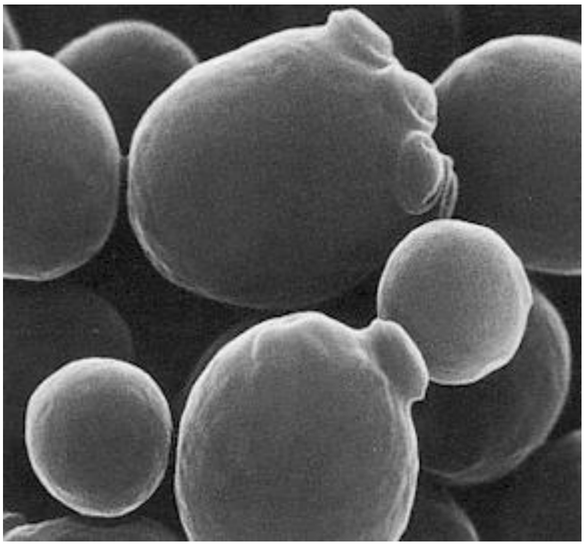


FIgure 3: *Saccharomyces cervisiae*

2.4. Brewing process

Brewing requires that the barley malt or the cereals used in the process will be under a malting process.

The **malting process** consists in soak the grains in drinking water to increase their moisture content, so that they can germinate. If the water level is quite low, there can be disintegrated and the becoming of high losses in the malting process, or worst, the dead of the germ. Once the soaking is produced, there is a drying process with hot air, the necessary to stop the germination, increasing the activity of the different enzymes present in the barley malt.

In the final stages of drying, the grain is toasted, producing a condensation of the possible groups of amino acids and proteins in the wort. The intensity of the toast depends on the type of beer we are brewing.

Finally, a degermination takes place, avoiding substances that can affect the taste and aroma of the beer, and it is stored.

In the brewing industry, the malting process is carried out **in situ**, as they have the necessary equipment to carry it out. Craftsman most of the time don't have the chance of having this type of infrastructures, so they are used to obtain the raw material already malted. (10)

After the malting process, the **wort production** takes place. First, the malt is grinded, obtaining a liquid that contain all the soluble substances necessaries for the brewing. In this process, nitrogen compounds are degraded, the starch and the phenolic compounds suffer oxidation reactions and precipitate with the proteins. For a high extract wort without haze is necessary a filtration and a wash of it.

After this step, it takes place a **cooking process** (inactive and sterilize the wort for coagulate proteins and tannins = color formation) and a hop process (gives bitter taste, smooth foam and give aroma).

The next step of the process is a **clarification,** a **cooling** and **aeration** of the product. Clarification decreases the haze doing a centrifugation or a filtration process. Once the wort is clarified it must be cooled for avoid oxidations and non-desirable flavors. It must be aired because the yeasts can have enough oxygen for its growing. (10)

Now it starts the fermentation step.

2.4.1. Alcoholic fermentation

Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation

Ethanol fermentation is a biological process which converts sugars such as glucose, fructose, and sucrose into cellular energy, producing ethanol and carbon dioxide as a side-effect. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process

The chemical equations below summarize the fermentation of sucrose ($C_{12}H_{22}O_{11}$) into ethanol (C2H5OH). Alcoholic fermentation converts one mole of glucose into two moles of ethanol and two moles of carbon dioxide, producing two moles of ATP in the process:

$$\mathbf{C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2}$$

Sucrose is a dimer of glucose and fructose molecules. In the first step of alcoholic fermentation, the enzyme **invertase** cleaves the glycosidic linkage between the glucose and fructose molecules.

$$\mathbf{C_{12}H_{22}O_{11} + H_2O + invertase \rightarrow 2\ C_6H_{12}O_6}$$

Next, each glucose molecule is broken down into two pyruvate molecules in a process known as glycolysis. Glycolysis is summarized by the equation:

$$\mathbf{C_6H_{12}O_6 + 2\ ADP + 2\ Pi + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2\ H^+}$$

The chemical formula of pyruvate is $CH3COCOO^-$. Pi stands for the inorganic phosphate.

Finally, pyruvate is converted to ethanol and CO2 in two steps, regenerating oxidized NAD^+ needed for glycolysis:

$$\mathbf{CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2}$$

Catalyzed by pyruvate decarboxylase:

$$\mathbf{H_3CHO + NADH{+}H^+ \rightarrow C_2H_5OH + NAD^+}$$

This reaction is catalyzed by alcohol dehydrogenase (ADH1 in baker's yeast).

As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.

The following figure shows the ethanol fermentation process:

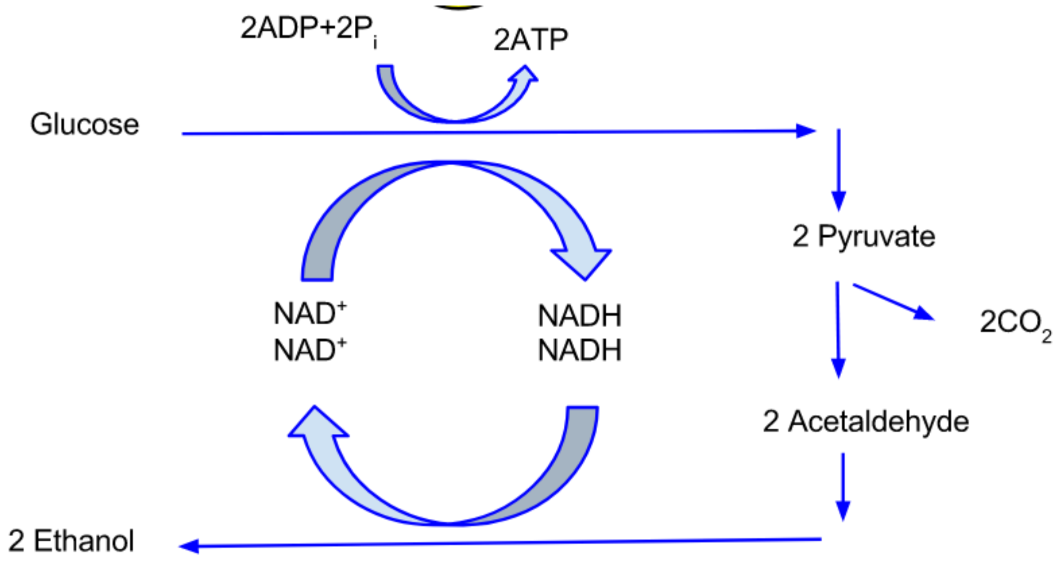


Figure 4: Ethanol fermentation

Once finished the fermentation, the product is not a real beer, because it contains particles in suspension, low carbonation, and immature taste, also microbiologically unstable. That's why beer suffers a maturation process (second fermentation in craft beers) or a filtration (industrial beers).

During the maturation process, in the industrial process, beer can transform into a pleasurable drink, and in the case of the industrial ones, is saturated with carbon dioxide, producing maturation with air absence and allowing the elimination of particles in suspension by a decantation. The moment for transfer the beer to the maturation batch is known by the diacetyl presence (compound originated in the fermentation), when it has a <0.1 mg/L concentration. (10)

The final processes of brewing are bottling (in craft beers, the final process is the second fermentation in the bottle) and biological stabilization or pasteurization (only in industrial beer). The materials normally used to packaging are glass or metal, because they are chemically inert.

Pasteurization happen in the bottle or can, but it also can be out, in the fermenter, and then packaged (totally aseptic). With this, it can be reduced the possibility of high levels of microorganisms that can affect the quality of the beer.

In craft beers, pasteurization is eliminated, maintaining the yeast activity (second fermentation). (10)

The next scheme shows in brief, the brewing process for an industrial beer:

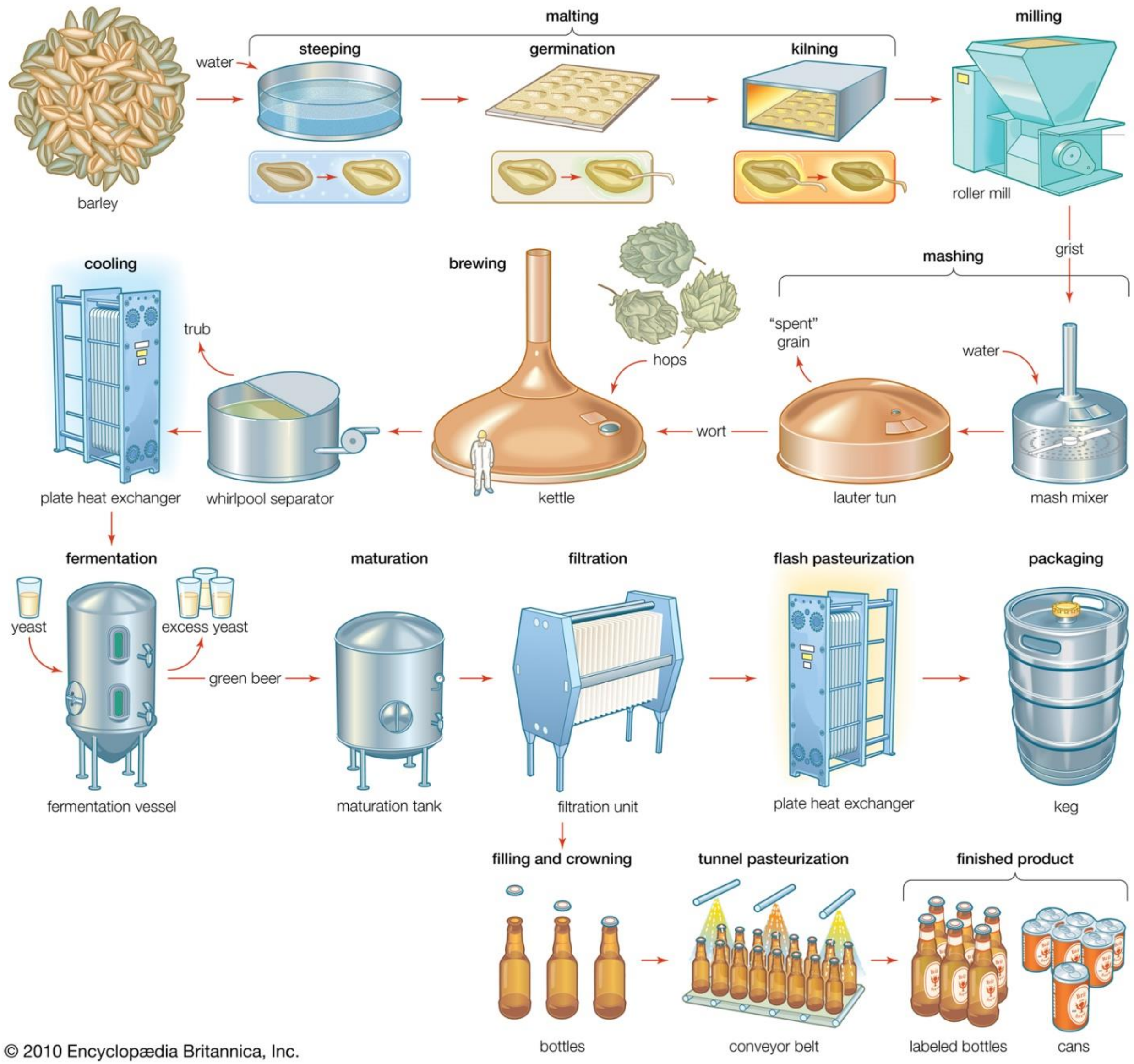


Figure 5: Industrial brewing process

2.5. Differences between industrial beers and craft beers

All type or beers, industrials and craft ones, are made with these basic ingredients: water, yeast, hops and malt of barley or other cereals. But, in fact, there are a lot of differences between an industrial beer and a craft beer.

The principal difference is the process and the technology used in the brewing process. Also, there is a huge difference in the quality of the raw materials and the recipe.

Industrial beers are produced with a basic formula, using economic processes and ingredients, with huge installations just for have more quantity of product in less time.

Craft beers is modified by the craftsman until he/she achieve a particular taste and smell, using traditional equipment, for have the better quality, without care a lot of the time. (7)

If we focus on the ingredients, industrial beers uses water, hops and yeast (and sometimes malt), and also, mixing this ingredients with cheaper ones like rice. Industrial ones add synthetic sugars or syrups just for accelerate the fermentation process. Odorants, stabilizers, filtering and clarifying agents, and enzyme preparations are added too.

FIgure 6: Common ingredients used in industrial beers and in craft beers

Craft ones just use natural products, in little quantities, avoiding long storages, because the craftsman doesn't tend to use additives or other substances. (4, 7)

On product conservation issues, industrial beers use the **pasteurization**, to ensure a longer beer life, but this process makes **loses of organoleptic characteristics** of the product, also, destroying the yeasts, stopping the chemical reactions. Also, industrial beers suffer a **filtration** step in their making process, eliminating microorganisms (clearer beers), avoiding a second fermentation in the bottle, generating less CO_2, and making necessary a gas injection.

In other way, craft beers don't use the pasteurization, obtaining a better taste and aroma and the yeast can keep doing its reactions. They don't suffer a filtration, obtaining turbid beers. Avoiding pasteurization and filtration, craft beers suffer a **second fermentation in bottle**, saturating the beer with carbonic gas and ethanol, resulting beers with higher graduation than the industrial ones. (4, 7)

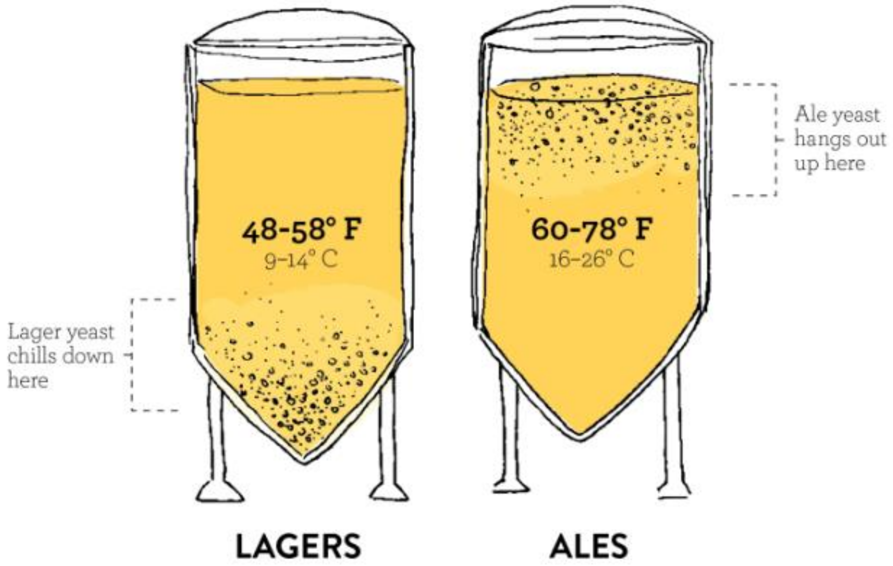


FIgure 7: Differences between fermentations in Ale and Lager beers

2.6. Beer styles

Many thousands of different beer brands are produced all over the world and they can be classified into different styles, depending on its elaboration and the region of the world they were created.

Beers are classified in three great styles according to their **fermentation** and, therefore, by the strains of yeasts used. They are called Ale, Lager and Lambic:

2.6.1. Ale beers

Ale is a type of beer brewed using a warm fermentation method, resulting in a sweet, full- bodied and fruity taste. Historically, the term referred to a drink brewed without hops.

As with most beers, “ale” needs the addition of a bittering agent to balance the sweetness of the malt and act as a preservative. Ale was originally bittered with gruit, a mixture of herbs or spices boiled in the wort before fermentation. Later, hops replaced gruit as the bittering agent

The yeasts that give to ale-style beers are of the genus Saccharomyces cerviciae. They are called high fermentation yeasts because they rise to the surface of the tank at the end of the fermentation and perform their activity between 14-26ºC. At this temperature, the yeast can produce significant amounts of esters and other secondary flavor and aroma products and the result is often a beer with slightly “fruity” compounds resembling those found in fruit such as apple, pear and banana. This style comes from Britain, Germany and Belgium. (5).

FIgure 8: Different Ale beers

2.6.2. Lager beers

Lager is a type of beer brewed at low temperatures, with a process named cool fermentation, and maturated in cold storage, between 9 – 14ºC. In its process is used a specific yeast, the Saccharomyces. pastorianus. It may be pale, golden, amber, or dark.

Lager is characterized by a clean taste and high levels of carbonation thanks to its particular brewing style. Lagers are typically 3-5% alcohol by volume. (5, 6)

FIgure 9: Different Lager beers

2.6.3. Lambic beers

Lambic is a type of beer with an old tradition: accounts dating from 1559 mention the production of Lambic "according to an old recipe". It is 30% wheat, and 70% malt spontaneous fermented beer seasoned with hops.

This wheaten beer can used to be brewed in the southwest of Brussels, because of the presence of specific wild yeast like Brettanomyces bruxullensis and Brettanomyces lambicus, but nowadays any brewer around the world can achieve the yeast and make its own lambic beer.

Lambic can be drunk direct from the barrel, but usually it is used as base for the elaboration of 6 other beers (Geuze, Faro, Kriek, Framboise, Pecheresse and Cassis). Lambic brewing is known by its spontaneous fermentation exposed to open air, and uses dry hop, avoiding bitter tastes. Lambic is a seasonal beer only brewed in the winter season (October-May). (5)

FIgure 10: Different Lambic Beers

2.7. Aspects to consider in brewing

In brewing is necessary to take account certain parameters to obtain a good product, with the organoleptic characteristics that are expected according to the recipe. Each brewer has its own recipes and particularities in the elaboration processes, but all of them consider a group of variables:

2.7.1. Density/Gravity

In the context of fermenting alcoholic beverages, gravity is the relative density compared to water, of the wort at various stages in the fermentation. This concept is used in the brewing and wine-making industries. Specific gravity is measured by a hydrometer, refractometer, pycnometer or an oscillating U-tube electronic meter.

The density of a wort is largely dependent of the sugar content of the wort. During the fermentation, yeast converts sugars into carbon dioxide and alcohol. The decline in the sugar content and the presence of ethanol (less dense than water) drop the density of the wort.

It is measured in Plato degrees (ºP). In wine industry is the equivalent to the Brix scale (14).

A Plato degree is the amount in grams of dry extract of the original must of the beer contained in 100 grams of this must at the temperature of 20 degrees Celsius (13). The more density, the more content of alcohol in the beer and more hops will be needed in the process. Dense worts need more time for fermentation and more maturation.

Figure 11: Common hydrometers used in brewing

2.7.2. pH

The pH is really important in the fermentation step. The pH levels during the different stages of the brewing process affect the extract potential, beer color, hot-break formation, foam stability, hop oil extraction, hop bitterness and **lauterability** in the beer. It is also important, considerate pH during storage as an inhibitor of bacterial growth, decreasing the pH value.

pH influence the yeast activity, for example, for *Saccharomyces cerevisiae*, the optimum pH oscillates between 4.4 and 5, being 4.5 the most suitable for it growing.

During the brewing process, the pH of beer suffers changes. Water from most municipal water sources will have a pH over 7 (because it is treated to prevent corrosion of pipes). When combined with crushed malt, the pH of the grain and water mixture drops considerably compared to the initial pH of the water alone.

The presence of other minerals within the brewing water can interfere with the pH decrease during the brewing process. Specifically, the carbonate (CO_3^{-2}) and bicarbonate (HCO_3^{3-}) ions (the ions associated with temporary water hardness) whom can act as buffers to pH decrease. These ions interact with water molecules to form hydroxyl ions (OH^-)

These extra OH^- ions will then react with any H_3O^+ ions that they happen to encounter and produce water molecules. This effectively removes the extra H^+ ions that are being generated by the brewing process and limits the natural pH decrease. This is why it is important to ensure that the ions responsible for temporary hardness are removed from the brewing water, especially when brewing light-colored beers.

During anaerobic fermentation, a part of the ethanol production, it can be generated organic acids like lactic acid, propionic acid and pyruvic acid.

pH in beer is usually measured with a common pH-meter (19).

2.7.3. Astringency

Astringency, as it's understood in sensory science, is generally described as a **rough, dry sensation** across the oral cavity. This sensation is caused by an interaction between various polyphenols (from the hops and malt) and certain proteins found in your saliva.

When these large and complex molecules interact, they assemble themselves into networks which are too large to stay in solution, and the lubricating properties of the salivary proteins are rendered inactive. This leads to the dry and rough mouth feeling which is detected by the trigeminal nerve (responsible for the tactile sensations of the face). This is caused by the presence of flavonoids, proanthocyanidins and **polyphenols**.

Astringency also can be caused by over-sparging, steeping the grains too long, and mash pH above 5.2, over hopping or an excessive grain boiling. Also can be a signal of oxidation of the beer. (20)

To control the astringent taste of a beer, it is necessary to take into account the maceration time of the malt or cereal, control the pH levels, and very important, control the amount of hops used in the recipe (28).

2.7.4. Bitterness

Bitterness is one of the four basic flavors and a desirable characteristic in beers. Is provided by compounds such as **humulones** or alpha acids from hops used during brewing. These acids undergo isomerization from both *cis-* and *trans-* isohumolone, which are responsible of the bitter taste of the beer.

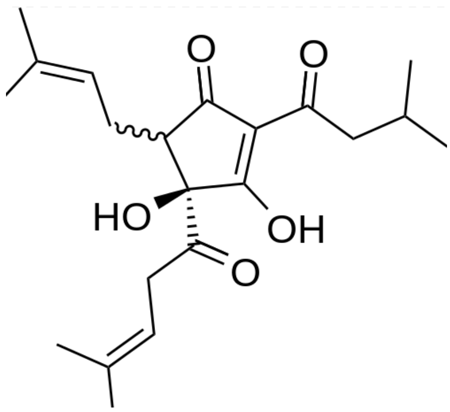
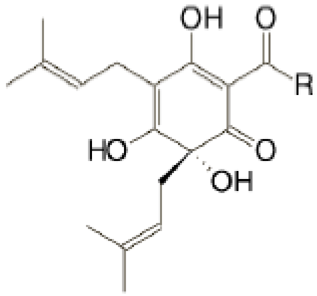


Figure 12: Isohumulone structure

It varies with the style. Bitterness is measured with IBU degrees (International Bitterness Unit) and it is equal to 1 mg of α-acids/L of beer, in other words, the amount of bitter substances diluted in the beer. The α-acids can be determined by conductimetric titration with a solution of lead acetate. (21)



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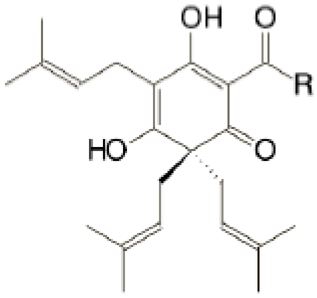

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Figure 13: α –acids from hops

2.7.5. Haze

After packaging, the majority of the haze problems are by the formation of **protein-polyphenol complexes** (tannins), forming colloidal particles.

During boiling and the cooling, the complex can precipitate. It is important for the characterization of the color and the amount of polyphenols in the beer. In oxygen presence, and **heavy metals** like copper or iron, polyphenols can be activated, reacting with the proteins forming this complex. (20)

Figure 14: Beer haze

2.7.6. Foam

Foam is a very prized quality in beer. A beer often tastes different when it's topped with head of foam, and this is due to surface active compounds that move into the bubble walls as they percolate to the top of the glass. Foam also carries a profound trigeminal sensation, that is, "taste" effects which are actually perceived physically

When beer foams, it is obviously due by the creation of bubbles. This phenomenon is referred to as **nucleation.**

The physics of nucleation as a whole isn't entirely understood, and there are a large group of proteins and smaller polypeptides (additional proteins) that can act as a group and individually as foam positive agents.

It is important to know that if the beer continues fermenting in the bottle, then it naturally carbonates and the foam is formed upon opening and/or pouring the beer. If the beer is pasteurized or filtered then the beer must be force carbonated using pressurized gas.

The density and longevity of the foam will be determined by the type of malt and adjunct from which the beer was fermented. Different mash schedules and cereal sources influence head retention. In general, wheat tends to produce larger and longer-lasting heads than barley.

While the actual foam activity of beer depends on the presence of carbon dioxide, it is the surface-active materials like amphipathic polypeptides from malt that determine size, shape and length of the foam. (20)

Figure 15: Beer foam

3. Parameters to analyze

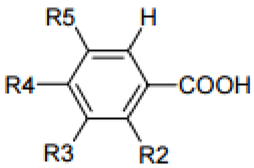
3.1. Polyphenols

Polyphenols are a structural class of mainly natural, but also synthetic, organic chemicals characterized by the presences of large multiples of **phenol structural units**. The number and characteristics of these phenol structures underlie the unique physical, chemical and biological properties.

They are secondary metabolites naturally present in plants. They have great importance for the food and drink products derived from plants, since these compounds are responsible for their organoleptic properties. As a consequence, they are closely related to the quality of such products, which makes their analysis considerably interesting (Robbins, 2003).

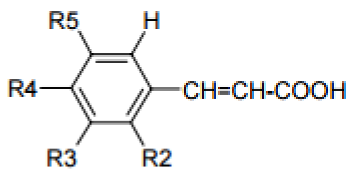
In the case of beer, it contains a complex mixture of phenolic compounds extracted from malt and hops which have been shown to have useful antioxidant properties (Goupy, 1999). Moreover, three groups of polyphenols are responsible for the beer flavor and physical stability (foam and haze).

Simply polyphenols derived from hydroxybenzoic acid (**gallic acid**, protocatechuic acid, etc.) and hydroxycinnamic acids (ferulic acid, caffeic acid, etc.) are extracted mostly from malt, but are also present in small amounts in hops. The final content of phenolic components of beer depends on both the raw materials and the brewing process. (20)



R_2	R_3	R_4	R_5	Name of the acid
H	H	H	H	benzoic
OH	H	H	H	salicylic
H	OH	OH	H	protocatechuic
OH	H	H	OH	gentistic
H	OH	OH	OH	gallic

Figure 16: Phenolic acids derived from benzoic acid



R_2	R_3	R_4	R_5	Name of the acid
H	H	H	H	cinnamic
H	H	OH	H	*p-coumaric*
H	OCH_3	OH	H	ferulic
H	OH	OH	H	caffeic

Figure 17: Phenolic acids derived from cinnamic acid

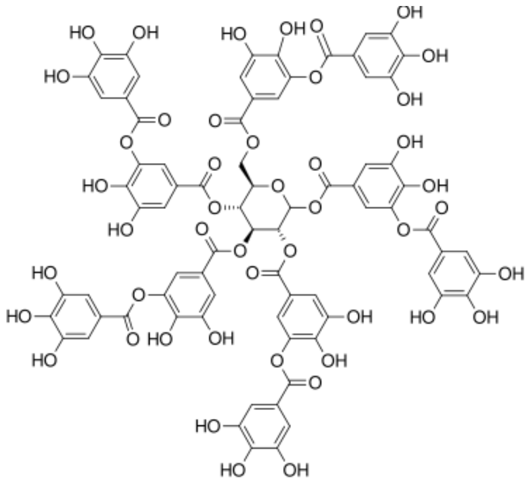


Figure 18: Tannic acid

Polyphenols are often macromolecules deposited in cell vacuoles. Polyphenols always have heteroatom substituents, like hydroxyl groups; ether and ester linkages are common, as various carboxylic acid derivatives.

They are molecules owing their UV/Vis absorptivity to aromatic structures with large conjugated systems of pi electron configurations, they also have fluorescence properties. They are reactive species toward oxidation.

Polyphenols also characteristically possess a significant **binding affinity for proteins**, with lead the formation of soluble an insoluble protein-polyphenol complexes, in the case of beverages like **beer.** (17)

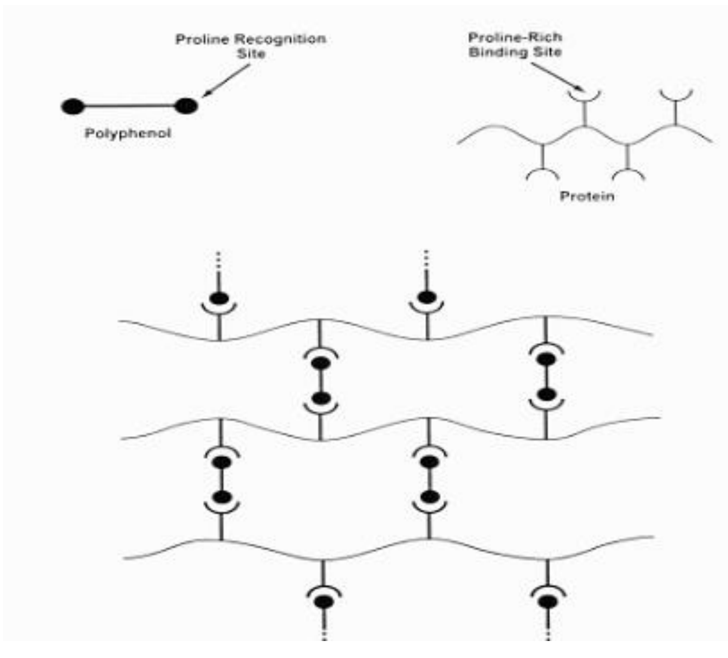


Figure 19: Siebert’s model for protein – polyphenol interaction in Beer

These structures are abundant micronutrients in our diet, and evidence for their role in the **prevention of degenerative diseases** such as cancer and cardiovascular diseases is emerging. (18) They are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens.

Polyphenols undeniably behave as antioxidants in food systems and hence improve food and beverage functionality in terms of foamability, oxidative stability and heat stability. However, degradation and de-polymerization of flavonol oligomers and polymers have been known to occur during food and beverage processing and storage.

The fate of polyphenols during the brewing, processing and aging must also therefore be regarded. Polyphenols losses due to haze formation, adherence to yeast cells during fermentation and removal by filtration (industrial beers) media are inherent, but what fate is bestowed upon polyphenols during aging? Unfortunately, the fate of polyphenols on packaged beer remains unresolved. During beer aging, polyphenols are gradually degraded by oxidative mechanisms into other spices whose chemical reactivity remains largely unknown (Aron, Patricia, 2010) (22).

3.2. Proteins

3.2.1. Proteins composition and structure

Proteins are organic molecules that are essential to life. They perform several important roles as structural components of cells, and when they are in a water solution; their function is being enzymes that catalyze reactions. Organic catalysts initiate chemical reactions without being part of them.

Most proteins fold into unique 3-dimentional structures. He shape into which a protein naturally folds is known as its native conformation. Although, many proteins can fold unassisted, simply through the aid of molecular chaperones to fold into their native states. Proteins are made by the following structures:

- **Primary structure**:

Is the amino acid sequence. Is when we call this structure a **polypeptid.**

- **Secondary structure:**

The polyamides are stabilized by hydrogen bonds. The most common examples are the **α-helix** and **β-sheet**. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.

- **Tertiary structure:**

The overall shape of a single protein molecule; the spartial relantionship of th secondary structures to another. Tertiary structure is generally stabilized by nonlocal interactions, most comonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds and even posttranslational modifications. The tertiary structure is what **controls the basic function of the protein**.

- **Quaternary structure:**

The structure formed by several protein molecules (polypeptide chains), usually called protein subunits in this context, which function as a single protein complex (27).

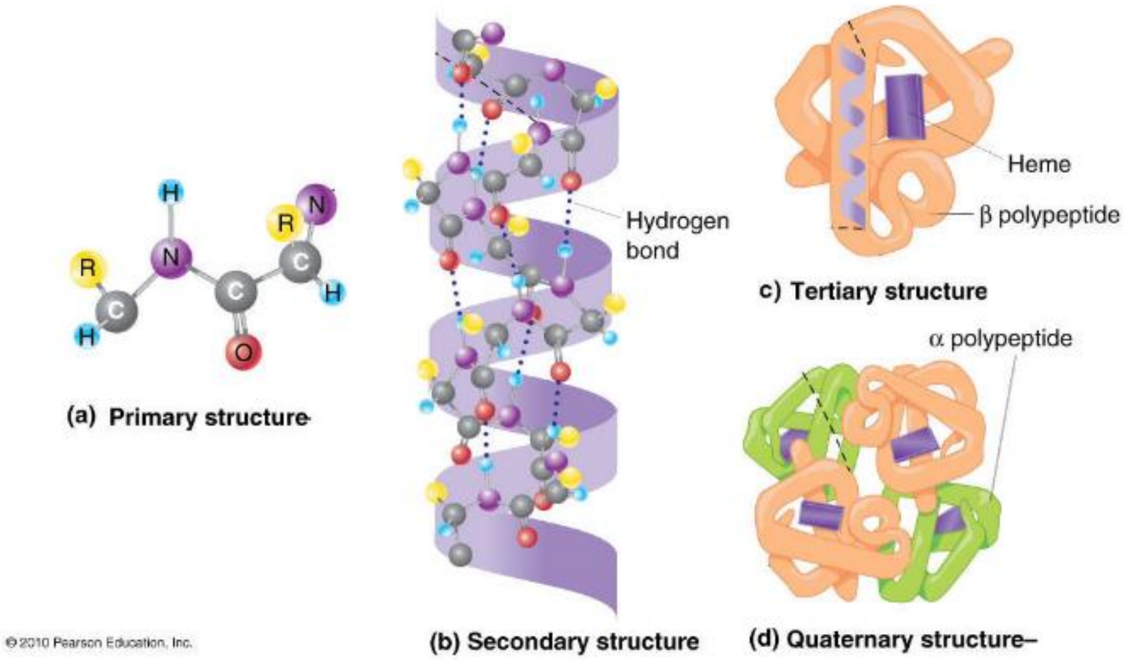


Figure 20: Protein structures

Polypeptides are small proteins of fewer than 100 amino acids; highly complex, large-molecular proteins may have many thousands of polypeptides. Based on these structural characteristics, some proteins, such as collagen, form the fiber animal tissues, whereas other proteins, those with very complex shapes, may become enzymes. (27)

3.2.2. Protein role in brewing

In brewing, we found a complex relationship with proteins, which are looked upon as both a curse and a blessing.

The nitrogenous compounds that concern the brewer start with uptake form soil in the grain fields and then follow through the entire brewing process into the beer drinker's glass.

Protein management throughout the malting and brewing process, therefore, is an essential part of making good beer, or, in fact, any beer at all.

Proteins, in the form of enzymes, are the key organic catalysis that break down components on raw barley and make them process compliant for such operations as lautering and filtration and finally suitable for yeast to metabolize during fermentation.

Without enzymes doing their catalytic work, we could not break down grain starches into sugars and yeast would, quite literally, have nothing to ferment. Enzymes convert large-molecular starches into yeast-fermentable sugars; they reduce large-molecular proteins into smaller ones, including amino acids, which are essential for healthy yeast growth and development.

Enzymes perform these functions at various stages in the malting plant and in the mashing vessel. The brewer will use the mashing profile to break down certain proteins and leave other intact. The breakup of large-molecular proteins reduces mash viscosity and thus enhances the ability of the brewer to extract sugars through the grain bed during lautering.

As malt and other grains become wort, proteins take on other roles, and the brewer begins to attempt to manipulate the protein profile of both the wort and the finished beer. The boiling of the wort coagulates and precipitates proteins that, left intact, would make the resulting beer opaque, viscous and unstable. In the kettle and whirlpool, proteins leave the wort as granular sediment called **turb**.

Later, after the wort is cooled more protein will sediment out during the **cold break**.

Cold break is the name for all the crud that precipitates out the solution when you rapidly cool wort after the boil. A good cold break means the appearance of little flecks floating around within otherwise clear wort. This flecks consist of malt proteins, hops matter and malt tannins (polyphenols).

The composition of the cold break depends, naturally, on the composition of the raw ingredients used to prepare the wort (well-modified malts and under modified malts make for different cold breaks), but it also depends on your chosen mash schedule.

In industrial beers, brewers may hope to avoid protein-derived hazes and sediments in beer that is destined to be filtered bright, but he must not go too far.

Beer without proteins would have little body or mouthfeel; it would taste thin and empty. The sturdy crown of foam that is prozed on most types of beer is largely created by tactile and visual texture – foamless, wan, and unattractive. (26)

To become surface active, these barley proteins undergo a structural maturation in the malting process, and unfolding on heating during the brewing process. This modifications or steps in brewing increase the amphiphilic[1] character of the proteins and their spreading behavior at the gas-liquid interfaces.

The proteins of this beverage belonging to the category of alcoholic beverages are formed by amino acids such as aspartic acid, glutamic acid, alanine, arginine, cysteine, phenylalanine, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, tyrosine, threonine, tryptophan and valine. These amino acids combine to form the proteins of beer (25)

In beer we also can found two types of proteins, both important in the style and precedence of the beer (craft or industrial); **Haze–Active Proteins** and **Non-Haze-Active Proteins**.

[1] Amphiphilic: chemical compound possessing both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties

- **Haze-Proteins (HA)**

Beer HA protein is derived from hordein, which is a problem. Prolamins are defined as an insoluble protein in water but soluble in the 70% aqueous ethanol. So, by definition, native prolamins have limited water solubility. Hordein that is degraded by proteases during malting and mashing in somewhat water soluble and certainly the part that survives into beer is soluble in dilute alcohol solution. Gliadin, the wheat prolamin, is commercially available, while hordein is not.

- **Non-Haze-Proteins (Non-HA)**

Beer with Non-HA-Proteins is poor in proline, a protein important for the formation of the protein-polyphenol binding. This characteristic is important for clear beers, without haze. These types of proteins are perfect for brewing at industrial level, to make less complicated filtrations. (24)

4. Methodology

4.1. Sample description

Four different styles of craft beers and four types of industrial beers were studied in the present work. Each craft beer had its equivalent industrial one in order to evaluate the effect of beer-making process (craft or industrial) on the beer chemical characteristics. The four styles of beer had different elaboration process and organoleptic characteristics, which are shown below:

4.1.1. Industrial brewing beers

We have chosen as object of analysis the most popular beers of the Spanish brewing house Damm®:

- **Estrella Damm**®

Low fermentation industrial beer. Blonde **lager** style. Made with barley malt and rice. It has poor malt and hop aroma with a lightly fruity touch. Very pale straw color with nonresistant white foam. Fresh and dry at taste and lightly bitter. High levels of carbonation provide it a slight acidity.

- **Voll-Damm**®

Low fermentation industrial beer. **Märzen** (German) style. Made with malt, rice and corn. Toasted aroma with a reddish orange color. Deep, crystalline, shiny with solid white foam. Sweet taste (due to rice and corn Æ high amount of sugar) and dry ending. Creamy and carbonated.

- **Bock Damm ®**

Low fermentation industrial beer. **Dark Lager** style. Made with pale malt, Munich malt and roasted chocolate malt. Dark brown color with strong foam. Toasty and sweet. Carbonated

- **A.K Damm ®**

Low fermentation industrial beer. **Pilsen** style. Pure malt beer. Golden beer with white foam. Soft taste. Carbonated

4.1.2. Craft beers

- **Budejovicky Budvar®**

Low fermentation craft beer. Blonde **lager** style. Made with barley malt. Fresh and light taste, quite dry. Very pale and golden. Not so foamy. Low-carbonated

- **Löwenbräu Oktoberfestbier®**

Low fermentation craft beer. **Märzen** (German) style. Made exclusively by malt. Brilliant golden beer with white foam. Really aromatic with touches of honey and quite citric. Low carbonated.

- **Budejovicky Budvar Dark Lager®**

Low fermentation craft beer. **Dark Lager** style. Made with pale malt, Munich malt and caramel malt. Black beer, opaque with plenty of liveliness and a lasting beige foam. The aroma is lightly toasted like chocolate with touches of lemon. Low carbonated.

- **Krombacher Pils®**

Low fermentation beer. **Pilsen** style. Pure malt beer, Bright golden beer with white foam. Barley flavor with floral and apple touches. Low carbonated.

4.2. Methods

4.2.1. Total polyphenol quantification using the Folin-Ciocalteu

4.2.1.1. Fundaments of the method

The Folin-Ciocalteu assay is used as a measure of the content of phenolic compounds in vegetable products. It is based on the fact that phenolic compounds react with the Folin-Ciocalteu reagent, at basic pH, resulting in a blue color susceptible to be determined spectrophotometrically at 765 nm. This reagent contains a mixture of sodium tungstate and sodium molybdate and phosphoric acid that reacts with the phenolic compounds present in the sample.

The phosphomolybdotungstic acid (formed by the two salts in the acid medium), with a yellow color, when is reduced by the phenolic groups gives a complex of intense blue color, whose intensity is the one that we measured to evaluate the content of polyphenols in our sample. (18)

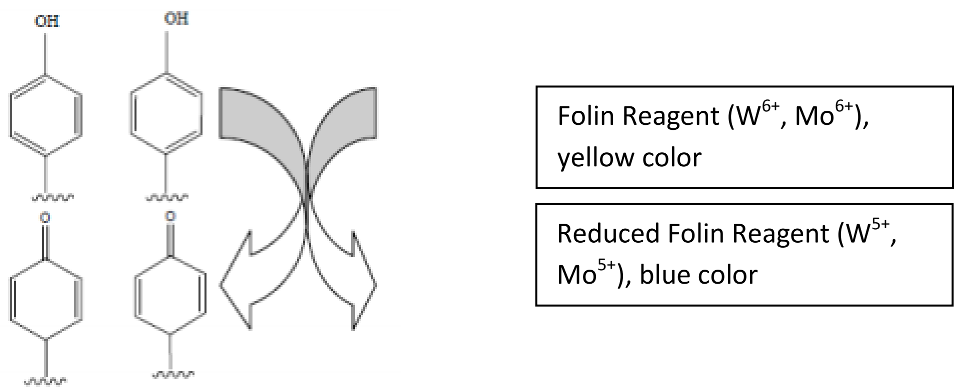


Figure 21: Mechanism of action of the Folin-Ciocalteu reagent

The mechanism used is a redox reaction, so it can also be considered as a method of measuring the total antioxidant activity in a sample of food. The oxidation of the polyphenols present in the sample to ketones.

The concentration of polyphenols is determined thanks to a calibration curve performed with a gallic acid solution. This method is really sensitive and precise.

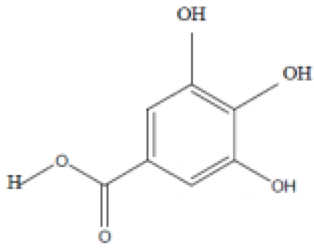


Figure 22: Gallic acid

4.2.1.2. Materials and reagents

- 25 mL of volumetric flasks
- Volumetric pipets
- Array Diode spectrophotometer
- Folin Ciocalteu reagent
- Sodium carbonate 20 % (m/v)
- Gallic acid
- Different beer samples (mentioned before at 4.1.1 and 4.1.2)

4.2.1.3. Procedure

4.2.1.3.1 Calibration curve

In order to get the calibration curve, first, we prepare a 0.45 % (m/v) of an acid gallic aqueous solution in a 100 mL volumetric flask, (S1). From this one (S1), we take 1 mL and we dilute it to a final volume of 50 mL with deionized water in a 50 mL volumetric flask (S2).

Now, from S2 we take 5 standard solutions, each one of 1,2,3,4 and 5 mL.

Volume (mL)	Concentration (mg/L)
1	3.6
2	7.2
3	10.8
4	14.4
5	18

Table 1: Standard solutions for the Folin method

In order to make the calibration curve, we must follow these steps:

- In a 25 mL volumetric flask, add a volume of the gallic acid solution (1,2,3,4 or 5 mL)
- Add 1 mL of the Folin-Ciocalteu
- Add 3 mL of the sodium carbonate solution 20 % (m/v)
- Add deionized water until 25 mL (limit of the flask)
- Shake the solutions using the vortex
- Let them rest for 1 hour, for the color development
- Do the lecture of the samples against a blank
-

Do the analysis 2 times, for better results

The results given by the spectrophotometer, at 765 nm, are:

Concentration (mg/L)	**Absorbance**
0	0.0016
3.6	0.2792
3.6	0.2787
7.2	0.5432
7.2	0.5439
10.8	1.0406
10.8	1.0423
14.4	1.2700
14.4	1.2716
18	1.6009
18	1.6031

Table 2: Absorbance results – Standard solutions (Folin)

Plotting the absorbance, in function of the concentration, we obtain the following regression line:

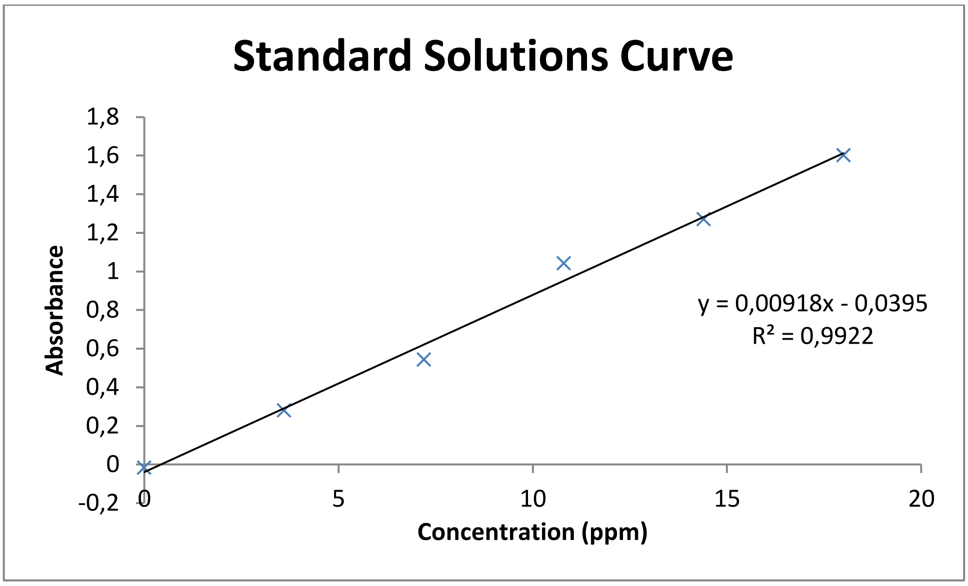


Figure 23: Standard solutions curve for the Folin-Ciocalteu Method

As we can see, the results are acceptable, viewing the R given by the equation; we see its value is 0.9922, value that can be accepted indicating a good performance of the analysis.

Figure 24: Standard solutions and their colors

4.2.1.3.2 Analysis of the samples

For the samples, we repeat the same process with the calibration solutions, unless, we only take 1 mL of each beer.

Once we do the analysis of the beers, we can obtain the results using the resulting equation of the calibration curve obtained before.

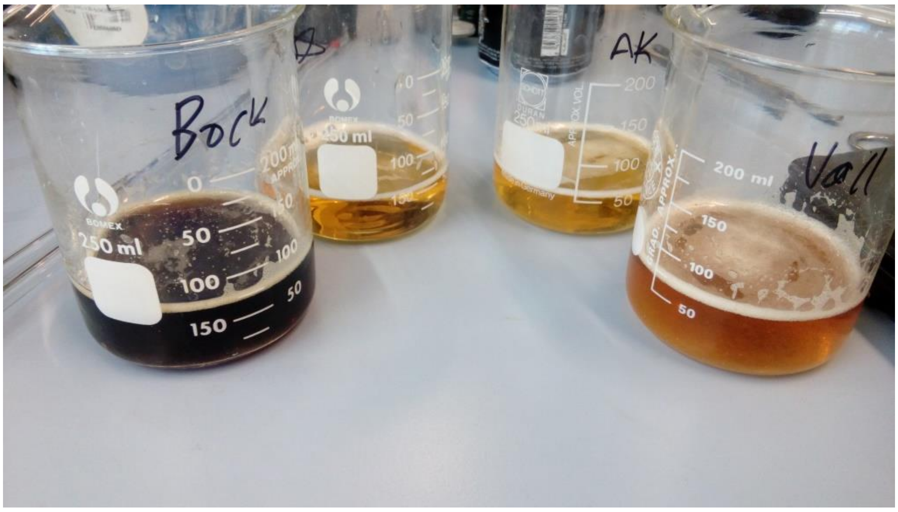


Figure 25: Beer samples

4.2.1.4. Results and discussion

In the following table (Table 3) we can see the concentration in mg/L of polyphenols of each beer we analyzed:

Type	Industrial		Craft	
	Name	**Concentration (mg/L)**	**Name**	**Concentration (mg/L)**
Lager	Estrella	130	Budejovicky Budvar	179
Märzen	Voll Damm	211	Löwenbräu Oktoberfestbier	235
Dark Lager	Bock Damm	206	Budejovicky Budvar Dark Lager	232
Pilsen	A.K Damm	199	Krombacher Pils	205

Table 3: Polyphenolic content of the beers

As we can see, the eight beers studied in this work have concentrations of polyphenols between 130 (Estrella Damm) and 235 mg/L (Löwenbräu Oktoberfestbier), these values are considered to be correct comparing with those of the literature. (21, 22)

Regarding craft and industrial beers, craft beers always exhibit higher concentration of polyphenols than their corresponding industrial ones for all the styles.

The fact that the beers present a certain polyphenolic concentration can be due to their not so aggressive process of elaboration, compared with the industrial and the craft brewing explained before.

The craft samples have higher polyphenol content due to the treatment received by the product in the brewing process, thus conserving most of the phenolic compounds, who come from hops, so the difference between these samples may reside in the quantity or variety of hops and in the processed, taking into account their types of conservation and ingredients. The quantity of yeast added to the sample can also influence in the polyphenolic content.

It is associated with yeast because it has the capacity to generate antioxidant organic compounds during the fermentation processes and when pasteurization is not performed, during the secondary fermentation in the bottle, it could increase the antioxidant capacity of the beer. (8)

Thus, regardless of the style of beer, craft beers outnumber, and in large values, industrials in the fact of having a higher concentration of polyphenols.

Our results can be compared with the ones obtained by Nakamura (2012), and also can be compared with the research made by Piazzon in 2010 for Italian beers, which have ingredients similar to those elaborated in the Iberian Peninsula. (21, 22)

In reference to the style of beer, we can see that in the samples studied here, the style with higher polyphenol content is the **Märzen**, in both, craft and industrial beers (Voll-Damm and Löwenbräu Oktoberfestbier). We can see its values are 211 mg/L for Voll-Damm and 235 mg/L for Löwenbräu Oktoberfestbier.

Acording to Piazzon (2010), for Märzen beers, using also the Folin- Ciocalteu method, the content range oscillates between 48 and 396 mg/L, and if we compare our results, we can see our sample falls into this range.

Dark Lager is the second style with more polyphenolic content, also in both types of beers (Boch Damm and Budejovicky Budvar Dark Lager). We can see its values are 206 mg/L for Boch Damm and 232 mg/L for Budejovicky Budvar Dark Lager.

Comparing with the results obtained by Nakamura (2012) and Piazzon (2010), the range content for Dark Lagers oscillates between 125 and 544 mg/L, so we can see our results are also into this range. It is necessary to say that the two beers have similar manufacturing processes unless the pasteurization step in the industrial brewing process.

The third style with bigger polyphenolic content is the **Pilsen**, also in both types of beers (A.K Damm and Krombacher Pils). We can see its values are 199 mg/L for A.K Damm and 205 mg/L for Krombacher Pils.

Comparing our results and the ones obtained by Nakamura (2012) and Piazzon (2010), the Pilsen range content oscillates between 54 and 460 mg/L but in this case, he uses the using Cu (II)/neocuproine complexes method. Piazzon uses the Folin-Ciocalteu method for the Pilsen beer and its results are quite similar to the Nakamura's (about 60 an 500 mg/L). So, we can consider our results as acceptable.

And finally, **Lagers** have the lowest polyphenolic content, also in both types of beers (Estrella Damm and Budejovicky Budvar). We can see its values are 130 mg/L for Estrella Damm and 179 mg/L for Budejovicky Budvar.

Comparing our results with Nakamura (2012) and Piazzon (2010), Lager beers have values ranging between 48 and 396 mg/L, the same range as the Märzen, unless their brewing process. We can see our results are into this range too.

As mentioned above, yeast has a very important role in the presence of polyphenols in beers, in addition to the fact that craft beers receive a more traditional treatment and do not need the use of additives or preservatives.

So, the fact that the craft samples have values greater than the industrial ones is due to its processes of elaboration and its ingredients.

4.2.2. Total protein quantification using the Bradford method

4.2.2.1. Fundamentals of the method

The Bradford protein assay (1978) is one of several simple methods commonly used to determine the total protein concentration of a sample.

The method is based on the proportional binding of the dye Coomassie to proteins. Within the linear range of the assay (~5-25 mcg/mL), the more protein present, the more Coomassie binds.

Furthermore, the assay is colorimetric; as the protein concentration increases, the color of the test sample becomes darker. Coomassie absorbs at 595 nm. The protein concentration of a test sample is determined by comparison to that of a series of protein standards known to reproducibly exhibit a linear absorbance profile in this assay.

Although different protein standards can be used, we have chosen the most widely used protein as our standard: Albumin

The method has been widely applied to biological samples, like food and drink samples. The polypeptides measured with this method are aromatic (phenylalanine, tyrosine and tryptophan) and basic (arginine, histidine and lysine)

The method can be applied directly to the brewing samples with some success, and it has been reported to directly asses high-molecular-weight proteins in beer. (25)

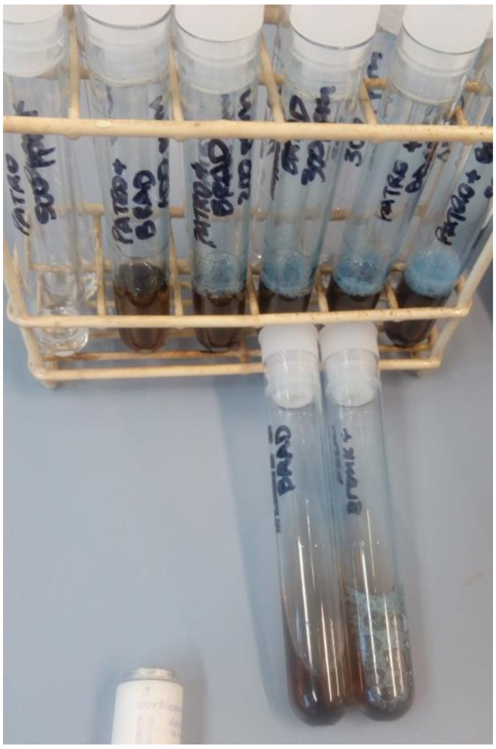


Figure 26: Standard solutions and samples with the Coomassie solution

4.2.2.2. Materials and reagents

- Coomassie Brilliant Blue reagent
- BSA (Bovine Serum Albumin)
- Deionized water
- Volumetric pipets
- Array diode spectrophotometer
- glass buckets
- test tubes
- Different beer samples (mentioned before at 4.1.1 and 4.1.2)

4.2.2.3. Procedure

- **Calibration curve**

In order to make the calibration curve, we must follow this process:

- Make a stock solution of Albumin of 5000 ppm (S1)
- From S1, make a 1/10 solution for have a 500 ppm solution (S2) to make a calibration curve with the following concentrations: 100, 200, 300, 400 and 500 ppm
- Homogenize the samples
- Take 0.1 mL from each one in a tube test
- Add 3 mL of the Coomassie Blue Reagent (Bradford Reagent) and homogenize them
- Let it rest 10 minutes
- Determine the absorbance with the spectrophotometer at 565 nm

Concentration (ppm)	Sol 500 ppm (ml)	Water (ml)
0	0.0	1.0
100	0.2	0.8
200	0.4	0.6
300	0.6	0.4
400	0.8	0.2
500	1.0	0.0

Table 4: Calibration curve concentrations

The next curve represents the calibration curve obtained with the spectrophotometer:

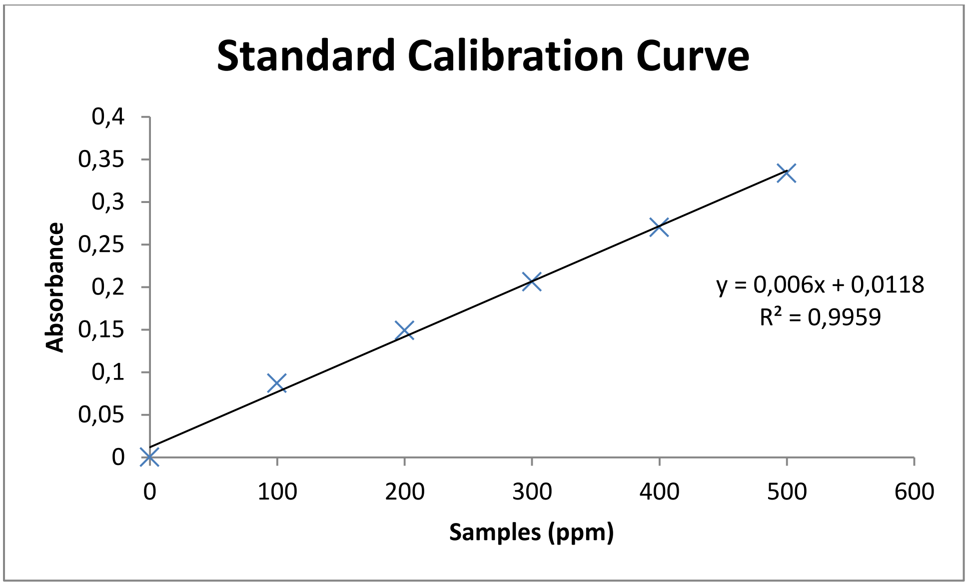


Figure 27: Standard solutions curve for the Bradford method

As we can see, the results are acceptable, viewing the R given by the equation; we see its value is 0.9959, indicating a good performance of the analysis.

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- **Analysis of the samples**

In order to analyze our samples we have to follow the next:

- Take 1 mL of the beer sample in a test tube and centrifuge it in the vortex at 3500 rpm for 15 seconds
- Separate the resulting foam with a pipette, leaving just the liquid in the test tube
- Add 1 mL of a 0.1 M NaOH solution and shake it in the vortex again for about 15 seconds
- Put the samples in a hot water bath for 5 minutes
- Take from every sample 0.1 Ml and put it in another test tube
- Add 3 mL of the Coomassie Blue Reagent (Bradford Reagent)
- Shake the tubes in the vortex for 10 seconds
- Determine the absorbance with the spectrophotometer at 565 nm

For the protein quantification, as we did with the polyphenol qualification, we focus on the equation obtained from the standard calibration curve. We express the results as mg of protein/L of H_2O, because, the calibration curve is expressed in mg of BSA/L of H_2O.

4.2.2.4. Results and discussion

In the following table (Table 5) we can see the concentration in mg/L of water of proteins of each beer we analyzed:

Type	Industrial		Craft	
	Name	**Concentration (mg/L H_2O)**	**Name**	**Concentration (mg/L H_2O)**
Lager	Estrella	100	Budejovicky Budvar	105
Märzen	Voll Damm	96	Löwenbräu Oktoberfestbier	99
Dark Lager	Bock Damm	101	Budejovicky Budvar Dark Lager	124
Pilsen	A.K Damm	93	Krombacher Pils	95

Table 5 : Protein content of the beers

As we can see, our beer samples have values between 93 (A.K Damm) and 124 mg/L (Budejovicky Budvar Dark Lager), It can be also observed that concentration of proteins are very similar between craft and industrial beers, except in the case of Dark Lager. For Lager, Marzen and Pilsen concentration values are very similar among styles and between craft and industrial elaboration process these values are around 100. Budejovicky is only sample that differs containing 124 mg/l that are a value sensibly superior than the other ones. This can be due to the quality of the raw materials or little differences in the process.

According to Sascha Wunderlich and Werner Back (2009), proteins in beer are really important, because if we focus in the drying/roasting of the malt in the craft brewing, the proteins coagulate and there are fewer losses, so this union is more efficient in roasted grains for a longer time, than in those that are partially or slightly roasted. This can be one of the reasons why craft beers have more protein content than industrial beers. (20)

Industrial beers are subjected to processed products which normally reduce protein levels to a large extent, such as the performance of various filtrates or clarifications, which is one of the reasons why additives and adjuvants must be added to form and stabilize the foam. In the craft beers, filtration is not carried out, so that not many nitrogen compounds are removed.

In addition to these processes, it is necessary to take into account the losses of protein by degermination of the grains during the malting and the consumption that the yeasts make during the fermentation

The protein content of the beer is an indication of the malt that was used for the elaboration, reason why the quality of the raw materials also influences. It is important to use cereals with optimal levels of protein, since low levels could not cope with yeast nutrition and, at excessive levels, would replace starch in the grain, producing a wort with little fermentable extract.

When there are too many nitrogenous compounds that are assimilable in the wort, the yeast cannot use them all and remain in beer, being a food for foreign microorganisms, posing a danger to the quality and conservation of beer. Due to the large productions, cereals that are cheaper than barley, such as rice, are used in the brewing industry and therefore are cereals that contain less protein, leading to beer with low levels.

Craft microbreweries are looking for a better quality of raw materials, so that the cereals used for malting, contain levels suitable to produce a wort that can cope with the losses of all processing, and still obtain a product end with some protein levels. For these reasons, the craft beers analyzed contain more protein than industrial beers. (20)

The results we have can be compared with the results obtained by Siebert, (2005) as he said, “the Bradford Method performed well in beer because it agreed well with results from electrophoresis of proteins followed by staining with the Coomassie Blue Reagent”. (24)

The results provided by Siebert (2005) after the analysis indicate that the content of proteins in a sample of beer, both craft and industrial, ranges **from 0 to 200 mg / L of H_2O, and our results fall in an average of 97.5 and 105.8 mg/L.**

The differences that we can consider, in terms of concentration levels, are if the proteins present are **Haze-active**, typical of craft beers (because they do not suffer filtration) and **Non-haze active**, own of industrial (these suffer filtration).

From our results, we can see in general, craft beers have more protein concentration than industrial Damm beers. These differences are due to the **quality of the raw materials** and to the **processes of elaboration** to which they are subjected, processes we described before.

So, if we compare our samples, the style with more protein content is the **Dark Lager**, in both type of beers (Boch Damm and Budejovicky Budvar Dark Lager). Its values are 101 mg/L for Bock Damm and 124 mg/L for Budejovicky Budvar Dark Lager. Our results fall in the range made by Siebert (2005)

Following Dark Lagers, we have the **Lagers** (Estrella Damm and Budejovicky Budvar) its values are 100 and 105 mg/L. As we can see, the minimum difference is because Budejovicky Budvar is a really pale beer, like Estrella Damm, but, the craft one doesn’t suffer filtration and doesn’t use rice in its brewing process.

Märzen are the third ones with more protein content in our samples (Voll-Damm and Löwenbräu Oktoberfestbier). The protein contents are 96 and 99 mg/L. As we see before,it happens the same with the Lager ones, their ingredients are so similar, but not their brewing process.

And, for last, we have the **Pilsen** ones (A.K Damm and Krombacher Pils). The protein contents are 93 and 95 mg/L. Again, we see there is a small difference in their concentrations.

As we can see, in this analysis, the results are quite similar between raft and industrial beers. It may be due to an improvement in the brewing process by the industrial ones, in order to preserve most of the proteins presents in beer. It also could be, in the craft brewing, an easily degradation of the proteins over time, maybe affected by the temperature and exposure, or the no utilization of preservatives in the brewing process. (20)

5. Conclusions

Polyphenolic content in the studied beers was ranging between 130 to 235 mg/L. These values agree with the results described in the literature for different types of beers.

The differences in content may be due to hops quality (richness in resin), filtering processes and the use of additives and adjuvants, which could reduce oxidation during storage. Yeasts can synthesize antioxidant organic compounds during fermentation, so the amount of brewer’s yeast could be essential to make a beer with greater antioxidant capacity.

Craft beer contains higher concentration of total than industrial beers.

Protein content is ranging between 93 and 124 mg/L. These values agree with contents reported previously. It is due to differences in the filtration processes that industrial beer receives or using cereals with lower protein content like rice or others. Also probably due to the destruction of the grains during the malting and the consumption that make the losses during the fermentation.

Protein concentrations are slightly higher in craft samples than in industrial ones. This can be due to the lake of filtration processes and using cereals with an optimal protein composition, just like the craft brewing does, and wort rich fermentable sugars.

To obtain a beer with high levels of protein a higher initial density is necessary, reducing or eliminating.

In short, craft samples have higher levels of polyphenols and proteins than industrial ones. This is interesting in relation to antioxidant properties and foam quality.

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